

Application No.: 10/500,240
Amendment Date: 22-Oct-10
Reply to Office Action of: 14-Sep-10

REMARKS/ARGUMENTS

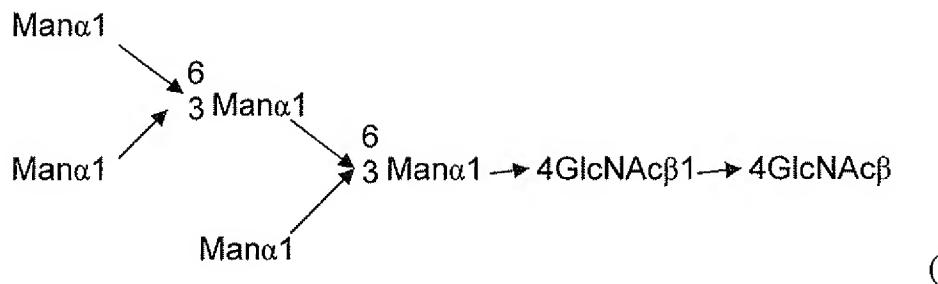
Claims 1-3, 6-12, 14-15, 17, 59, and 66-88 are pending.

I. Claim rejections 35 USC § 103

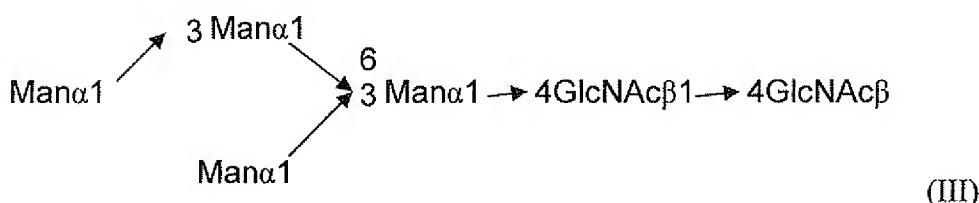
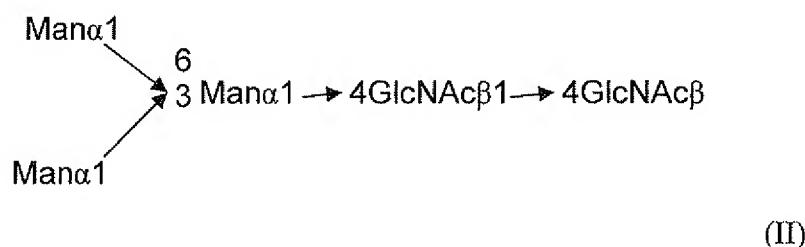
Claims 1-3, 61-12, 14-15, 17, 59, and 66-88 were newly rejected under 35 USC § 103(a) as allegedly being unpatentable over Nakanishi-Shindo et al., J. Biol. Chem. 368: 26338-26345 (1993) in view of Roy et al. Biotechnol. Bioprocess Eng. 5: 219-226 (2000), Contreras et al., U.S. Patent No. 6,803,225 (patented Oct 12, 2004) and Lin et al., Proc. Natl. Acad. Sci. USA 82: 7580-7584 (1985).

The applicants respectfully disagree that the currently claimed invention is prima facie obvious over the cited prior art. The applicants currently claimed invention is drawn to a method for producing a recombinant glycoprotein that have GlcNAcMan_XGlcNAc₂ core structures, wherein X is 3 or 4, in a unicellular or filamentous fungus host cell that lacks α 1,6-mannosyltransferase activity, the method comprising the steps of (1) diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure, and (2) introducing into the host cell one or more nucleic acids molecules encoding (i) an α 1,2-mannosidase catalytic domain fused to a targeting peptide that targets the endoplasmic reticulum (ER) or Golgi apparatus in the host cell, (ii) a GlcNAc transferase I (GnT I) catalytic domain fused to a targeting peptide that targets the ER or Golgi apparatus of the host cell, and (iii) the recombinant glycoprotein.

Nakanishi-Shindo et al. teach *Saccharomyces cerevisiae* that comprise mutations of the *OCH1*, *MNN1*, and *ALG3* genes and thus accumulated glycoproteins that have Man₅GlcNAc₂ or MangGlcNAc₂ N-glycans. These structures are shown in Fig. 4A and Fig. 3A, respectively, of Nakanishi-Shindo. Lin et al. was cited for its description of the cloning and expression of the human EPO gene in CHO cells. Roy et al. provides a description of their research plans for producing a recombinant *S. Cerevisiae* host cell capable of producing glycoproteins that have complex N-glycans and Contreras et al. describes a recombinant *Pichia pastoris* host cell that is capable of producing glycoproteins that have a GlcNAcMan₅GlcNAc₂ structure. Both Roy et al. and Contreras et al. are concerned with a recombinant yeast host cell that produces N-glycans that after α 1,2-mannosidase digestion have the Man₅GlcNAc₂ structure (I) as shown below



The Man₅GlcNAc₂ structure I is the usual substrate for GnT I. In contrast to the prior art, the currently claimed method produces *N*-glycans that after α 1,2-mannosidase digestion have a Man₃GlcNAc₂ structure (II) or Man₄GlcNAc₂ structure (III) as shown below



An unknown at the time of the applicants' currently claimed invention was whether a structure other than structure I would be an efficient substrate for GnT I in a unicellular or filamentous fugal host such as *Pichia pastoris*. Bobrowicz et al. (Glycobiol. 14: 757-766 (2004)) is a publication by several of the applicants, which describes use of the currently claimed method to produce glycoproteins that have complex *N*-glycans. Bobrowicz et al. (page 762, right col, second para.) states

A significant unknown at the outset of this project was in the substrate specificity of the GnT I enzyme expressed in yeast. The endogenous substrate for GnT I in all known higher eukaryotes is Man₅GlcNAc₂ (Figure 1A), to which GnT I adds a single GlcNAc in β -1,2 linkage on the 1,3 arm. However, in the scenario described here, the two mannoses normally transferred onto the upper 1,6 arm are

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eliminated by deletion of *ALG3*, requiring GnT I to act on a Man₃GlcNAc₂ structure following the action of α-1,2-mannosidase (Figure 1B). Analysis of several mammalian cell lines blocked in core assembly reveals that GnT I must act on a Man₃GlcNAc₂ structure to allow the generation of at least some complex N-glycans in these mutant cell lines (Kornfeld *et al.*, 1979; Lehrman and Zeng, 1989; Stoll *et al.*, 1982). Based on in vitro studies, some GnT I enzymes are better equipped to handle this assignment than others (Altmann *et al.*, 1993; Strasser *et al.*, 1999; Vella *et al.*, 1984; Zhang *et al.*, 2003). Although all GnT I enzymes favor the Man₅GlcNAc₂ structure, the human GnT I used in this study acts with reasonable efficiency on a Man₃GlcNAc₂ substrate in vitro, whereas some (such as insect or plant GnT I) enzymes are substantially less effective when compared to their natural substrate, Man₅GlcNAc₂. The results presented here demonstrate that human GnT I, when properly targeted to the yeast secretory pathway, is able to act with high efficiency on a Man₃GlcNAc₂ substrate.

An Information Disclosure Statement that includes Boborowicz *et al.*, and Kornfeld *et al.*, 1979; Lehrman and Zeng, 1989; Stoll *et al.*, 1982, Altmann *et al.*, 1993; Strasser *et al.*, 1999; Vella *et al.*, 1984; and Zhang *et al.*, 2003 (cited in the above quotation) is submitted concurrently with this response.

In light of the applicants uncertainty as to whether GnT I could efficiently transfer a GlcNAc residue to the mannose residue on the 1-3 arm of the Man₃GlcNAc₂ structure (II) or Man₄GlcNAc₂ structure (III) in a recombinant unicellular or filamentous fungus, the currently claimed method would not have been predictable over the prior art. There is no disclosure in the cited prior art that would suggest to a person of ordinary skill in the art to genetically engineer a host cell capable of producing human-like complex N-glycans by deleting or disrupting the *ALG3*, *ALG9*, or *ALG12* genes to produce glycoproteins in the host cell that have a structure in which it was uncertain the structure GnT I could efficiently act upon. Because of the uncertainty concerning whether GnT I could efficiently act upon the Man₃GlcNAc₂ structure (II) or Man₄GlcNAc₂ structure (III), the applicants' work which led to the currently claimed method would not have been the result of routine experimentation. Unlike the prior art, the currently claimed method provides a solution to the problem of genetically engineering unicellular and filamentous fungi to produce recombinant glycoproteins that have complex human-like N-glycans that does not involve the Man₅GlcNAc₂ structure (I). The currently claimed solution is both novel and inventive over the prior art.

In view of the foregoing remarks and amendments, it is believed that the previous grounds of rejections have been overcome and that the claims are in proper condition for allowance. Accordingly, Applicants respectfully request that all of the previous rejections be withdrawn and a Notice of Allowance be forwarded to the Applicants. The Examiner is invited

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to contact Applicants' Attorney at the telephone number given below, if such would expedite the allowance of this application.

CONDITIONAL PETITION

Applicants hereby make a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to effect this Conditional Petition.

Respectfully submitted,

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